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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 99/66877</b> <b>(43) International Publication Date:</b> 29 December 1999 (29.12.99)
<b>(21) International Application Number:</b> PCT/IL99/00352 <b>(22) International Filing Date:</b> 24 June 1999 (24.06.99) <b>(30) Priority Data:</b> 125097 25 June 1998 (25.06.98) IL <b>(71) Applicant (for all designated States except US):</b> AGRICULTURAL RESEARCH ORGANIZATION [IL/IL]; Volcani Center, P.O. Box 6, 50250 Bet Dagan (IL). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> ARAV, Amir [IL/IL]; Shlomit Zion Hamalka 59, 62266 Tel Aviv (IL). NITSAN, Zafira [IL/IL]; Hankin 23, 76354 Rechovot (IL). <b>(74) Agent:</b> FRIEDMAN, Mark, M.; Beit Samuellof, Haomanim 7, 67897 Tel Aviv (IL).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> DIETARY MANIPULATION TO INCREASE FERTILITY  <b>(57) Abstract</b>  The present invention provides a dietary supplement to increase fertility in an animal, the supplement comprising a $\omega$ -3 fatty acid-containing component. Furthermore, the present invention provides a method for increasing fertility in an animal, comprising feeding the animal a $\omega$ -3 fatty acid containing component. The $\omega$ -3 fatty acid containing component is preferably of plant, animal or single cell oil origin and is more preferably selected from the group consisting of whole fish oil, 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22:5 omega 3 (DPA) and 22:6 omega 3 (DHA) fatty acids and mixtures thereof, in an amount effective to increase fertility. Additionally, a method is provided for increasing fertility in an animal, by introducing a $\omega$ -3 fatty acid containing component into animal gametes, comprising feeding the animal a $\omega$ -3 fatty acid containing component, in an amount effective to increase fertility.		

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## DIETARY MANIPULATION TO INCREASE FERTILITY

### FIELD OF THE INVENTION

A natural, inexpensive feed supplement that increases concentration, motility and  
5 viability of sperm.

### BACKGROUND OF THE INVENTION

Over the last decade evidence has been mounting of declining fertility among  
domesticated animals and human beings. Sustained breeding for economically desirable  
10 traits is thought to be one of the major causes of reduced fertility and reproductive  
disorders in farm animals and poultry. A negative correlation was found between milk  
yield in dairy cattle and conception rate (Butler and Smith, 1989, see Appendix for full  
references). Mortality and reproductive dysfunction in broiler breeders was found to be  
related in part to increased feed consumption, resulting from selection for juvenile body  
15 weight (McCarthy and Siegel, 1983). A negative correlation was found between egg  
production in pigeons and selection for high body weight (Meleg and Horn, 1994).

Breeding of domesticated animals for high fertility traits has not provided a  
solution. Selection of fertility traits tends to be slow and increased fertility is often offset  
by reduction in other desirable traits. Alternative means of enhancing fertility and  
20 reproduction, such as optimisation of temperature, humidity, lighting and stocking density  
in animal houses or the use of hormones or dietary manipulation have only provided  
limited benefits.

The decrease in human fertility is most likely the result of many causes including  
marked changes in nutritional habits, which may be leading to deficiencies in specific  
25 nutrients.

All of the methods currently used for enhancing fertility involve treatments such as  
use of hormones, artificial insemination and in vitro fertilisation, involving skilled  
professionals and relatively high costs. They do not provide a complete solution for  
disturbed fertility.

30 The most important factors which affect fertilisation are sperm concentration and  
viability, egg viability and timing of fertilisation. At fertilisation eggs and sperm

membranes are fused and form an embryo. As a result membrane composition and quality are essential for fertilisation. Nissen et al., 1984, found in humans a high correlation between sperm concentration and membrane lipid composition. In the last decade many studies reported that dietary lipid composition may affect lipid composition of  
5 membranes, accompanied by changes in physical characteristics (Behar et al., 1989). An application of  $\omega$ -3 fatty acids can change the lipid composition of cell membranes and reduce heart and blood vessel diseases.

The phospholipids of mammalian spermatozoa display a highly characteristic fatty acid composition, with high proportions of docosahexanoic acid (DHA). In contrast to  
10 mammals, spermatozoa of avian species display extremely low concentrations of DHA in their phospholipids. Avian spermatozoa (domestic poultry) are characterised by high amounts of  $C_{20-22}$  polyunsaturated fatty acids (PUFA) of the  $\omega$ -6 series, whereas long chain fatty acids of the  $\omega$ -3 series predominate in mammalian spermatozoa. It appears that the 22:6 $\omega$ -3 present in mammalian spermatozoa performs an essential function in  
15 promoting optimal fertility. Commercial poultry production currently relies on compounded feeds that generally contain a vast excess of  $\omega$ -6 fatty acids over  $\omega$ -3 fatty acids. It is conceivable that the reported fatty acid composition of avian spermatozoa is a dietary induced displacement from the 'natural' pattern rather than a true phylogenetic difference from mammals.

20 Kelso et al., 1997, disclose the effect of dietary supplement of alpha linolenic acid on the phospholipid fatty acid composition and quality of spermatozoa in cockerel from 24 to 72 weeks of age. The fatty acid composition of the sperm phospholipid was shown to demonstrate a marked resistance to dietary manipulation. Their results suggested that the small increase in the proportion of  $\omega$ -3 fatty acids in the sperm phospholipids induced  
25 by enriching the diet with alpha linolenic acid is associated with a significant improvement in semen quality, such as an increase in spermatozoa concentration at 39 weeks of age.

Surai et al., 1997, disclose the relationship between the dietary provision of alpha tocopherol and the concentration of this vitamin in the semen of chicken and lipid  
30 composition. It was found that the concentration of alpha tocopherol in semen displays

only a limited responsiveness to manipulation by dietary means, but did provide beneficial changes in the lipid profile of the semen.

Ayala et al., 1977, disclose the effect of 22:6  $\omega$ 3 provided by dietary fish oil on the development of germinal tissue of rat testes and fatty acid composition of lipids. It was shown that at 7 and 9 weeks of age, development of germinal tissue in rats, which were fed fish oil was normal. The fatty acid composition showed a decrease in 22:5  $\omega$ 6 acid content and an increase in 22:6  $\omega$ 3 acid in triacylglycerol, phosphatidylcholine and phosphatidylethanolamine.

Nissen et al., 1983, disclose a linear correlation between concentration of docosahexanoic acid in semen and the spermatozoal density and number of motile normal sperm in humans.

Connor et al., 1997 disclose that sperm and retinal cells share important homologies. Both are rich in 22:6  $\omega$ 3 fatty acids. Patients with retinitis pigmentosa have low blood levels of 22:6  $\omega$ 3 fatty acids. It was shown that their sperm had a reduced motility and a much lower 22:6  $\omega$ 3 concentration than in normal subjects.

Coull et al., 1998, disclose the lipid and fatty acid composition of zona intact sheep oocytes. More than 50% of lipid in the oocytes was phospholipid or triglyceride. Long chain polyunsaturated fatty acids occurred infrequently.

The background art does not provide an effective method using dietary manipulation to increase sperm concentration, motility, viability and fertility or alternatively oocyte fertility. There is therefore a need for such a method, which is disclosed herein as the present invention.

#### SUMMARY OF THE INVENTION

The present invention provides a natural, safe, inexpensive feed supplement, which affects fatty acid composition of sperm membranes and increases the concentration, motility, viability and fertility of sperm in males such as turkey males. The present invention also provides a feed supplement, which increases oocyte or egg fertility, increases hatchability, decreases embryo mortality, increases follicles and increases egg number and egg quality in females. In addition, the present invention provides a way of

increasing sperm potency in all types of poultry and other farm animals and provides a feed supplement that is easy to use. Furthermore, the present invention enhances human fertility. The present invention also introduces  $\omega$ -3 fatty acids into gamete membranes and into oocytes.

5 In a first embodiment, the present invention provides a dietary supplement to increase fertility in an animal, the supplement comprising a  $\omega$ -3 fatty acid-containing component.

In a preferred embodiment the animal includes an agriculturally useful animal.

In a preferred embodiment the animal includes poultry.

10 In a preferred embodiment the animal includes a turkey.

In a preferred embodiment the animal includes a lower mammal.

In a preferred embodiment the animal includes an animal from the ovine species.

In a preferred embodiment the animal includes an animal from the bovine species.

In a preferred embodiment the animal includes a non-mammalian aquatic animal.

15 In a preferred embodiment the animal includes a human.

In a preferred embodiment the  $\omega$ -3 fatty acid containing component includes 18:3 omega 3 (LNA).

In a preferred embodiment the  $\omega$ -3 fatty acid containing component includes 20:5 omega 3 (EPA).

20 In a preferred embodiment the  $\omega$ -3 fatty acid containing component includes 22:6 omega 3 (DHA).

In a preferred embodiment the  $\omega$ -3 fatty acid containing component includes 22:5 omega 3 (DPA).

25 In a preferred embodiment the  $\omega$ -3 fatty acid containing component includes a mixture of at least two of the fatty acids, 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22:5 omega 3 (DPA) and 22:6 omega 3 (DHA).

In a preferred embodiment the  $\omega$ -3 fatty acid containing component includes fish oil.

30 In a preferred embodiment the  $\omega$ -3 fatty acid containing component is selected from the group consisting of fish meal, linseeds and purslane.

In a preferred embodiment the  $\omega$ -3 fatty acid containing component is a single cell oil source.

In a preferred embodiment the fertility is decreased embryo mortality.

In a preferred embodiment the fertility is oocyte fertility.

5 In a preferred embodiment the fertility is number of eggs.

In a preferred embodiment the fertility is quality of eggs.

In a preferred embodiment the fertility is hatchability.

In a preferred embodiment the fertility is number of follicles.

10 In a preferred embodiment the fertility is determined according to sperm concentration.

In a preferred embodiment the fertility is determined according to sperm viability.

In a preferred embodiment the fertility is determined according to sperm motility.

15 In a second embodiment the present invention provides a method for increasing fertility in an animal, comprising feeding the animal a  $\omega$ -3 fatty acid containing component, in an amount effective to increase fertility.

In a preferred embodiment of the method for increasing fertility, the fertility is determined according to sperm concentration.

In a preferred embodiment of the method for increasing fertility, the fertility is determined according to sperm viability.

20 In a preferred embodiment of the method for increasing fertility, the fertility is determined according to sperm motility.

In a preferred embodiment of the method for increasing fertility, the fertility is selected from the group consisting of laying rate, embryo mortality, number and quality of eggs, number of follicles or oocyte fertility.

25 In a preferred embodiment of the method for increasing fertility, the animal includes an agriculturally useful animal.

In a preferred embodiment of the method for increasing fertility, the animal includes poultry.

30 In a preferred embodiment of the method for increasing fertility, the animal includes a turkey.

In a preferred embodiment of the method for increasing fertility, the animal includes a lower mammal.

In a preferred embodiment of the method for increasing fertility, the animal includes a non-mammalian aquatic animal.

5 In a preferred embodiment of the method for increasing fertility, the animal includes a human.

In a preferred embodiment of the method for increasing fertility, the  $\omega$ -3 fatty acid containing component is selected from the group consisting of whole fish oil, 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA) fatty  
10 acids and mixtures thereof.

In a preferred embodiment of the method for increasing fertility, the  $\omega$ -3 fatty acid containing component is of animal origin.

In a preferred embodiment of the method for increasing fertility, the  $\omega$ -3 fatty acid containing component is of plant origin.

15 In a preferred embodiment of the method for increasing fertility, the  $\omega$ -3 fatty acid containing component is of single cell oil origin.

In a preferred embodiment of the method for increasing fertility, the  $\omega$ -3 fatty acid containing component is selected from the group consisting of fish meal, linseeds and purslane.

20 In a third embodiment the present invention provides a method for increasing fertility in an animal, by introducing a  $\omega$ -3 fatty acid containing component into animal gametes, comprising feeding the animal a  $\omega$ -3 fatty acid containing component, in an amount effective to increase fertility.

In a preferred embodiment, the animal includes an agriculturally useful animal.

25 In a preferred embodiment, the animal includes poultry.

In a preferred embodiment, the animal includes a turkey.

In a preferred embodiment, the animal includes a lower mammal.

In a preferred embodiment, the animal includes a non-mammalian aquatic animal.

In a preferred embodiment, the animal includes a human.

30 In a preferred embodiment, the animal gamete is a sperm.



In a preferred embodiment, the animal gamete is an oocyte.

In a preferred embodiment, the  $\omega$ -3 fatty acid containing component is selected from the group consisting of whole fish oil, 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA) fatty acids and mixtures thereof.

5 In a preferred embodiment, the  $\omega$ -3 fatty acid containing component is of animal origin.

In a preferred embodiment, the  $\omega$ -3 fatty acid containing component is of plant origin.

10 In a preferred embodiment, the  $\omega$ -3 fatty acid containing component is of single cell oil origin.

In a preferred embodiment, the  $\omega$ -3 fatty acid containing component is selected from the group consisting of fish meal, linseeds and purslane.

15 The term 'a gamete' hereinafter includes both male and female gametes, a sperm and an oocyte or egg respectively. The present invention can also be applied to oocytes. Throughout the specification where the term 'sperm' is used, oocyte can equally apply. Throughout the specification the term 'oocyte' as used herein refers to eggs and oocytes.

The term 'lower mammal' can be defined as any non-human mammal.

## 20 BRIEF DESCRIPTION OF THE DRAWINGS

FIG 1 shows the effect of dietary lipids on the sperm concentration (1), motility (11) and viability (111) of semen from tom turkeys.

FIG 2 shows the fatty acid profile of tom turkeys' sperm;

25 FIG 3 shows the effect of dietary  $\omega$ -3 fatty acids on volume, concentration and sperm number; and

FIG 4 shows the effect of  $\omega$ -3 fatty acid on the sperm motility and sperm concentration of bulls.

## DETAILED DESCRIPTION OF THE PRESENT INVENTION

30 The present invention provides a dietary supplement and method for increasing

fertility, which increases sperm concentration, motility, viability and fertility in males or alternatively increases egg fertility, increases hatchability, decreases embryo mortality, increases follicles and increases number and quality of eggs in females. Without wishing to be bound by a single mechanism, the fatty acids in the composition of the present invention may be absorbed through ingestion as part of the diet and may exert their effect by being incorporated into the sperm or egg membrane and affecting fatty acid composition of the sperm or egg membrane, respectively. This incorporation may lead to increased sperm concentration, motility, viability and fertility in the male or increased egg fertility, increased hatchability, decreased embryo mortality, increased follicles and increased number and quality of eggs in the female. Sperm concentration may be increased by metabolic or endocrine modification. The dietary supplement is at least one of the following or a mixture thereof of a  $\omega$ -3 fatty acid-containing component, 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA) fatty acids contained in  $\omega$ -3 fatty acids. The  $\omega$ -3 fatty acid-containing component can be of plant, animal or single cell oil origin. The composition of the present invention is envisioned as being added to the regular diet of agricultural animals, such as fowl, chicken, turkey, geese, mule ducks, ostrich and other poultry or even lower mammals, such as animals from the bovine and ovine species, non-mammalian aquatic animals and humans. The exact route of administration may depend on the animal e.g. for animals the supplement can be given as part of a feed, for humans the supplement can be given in tablet form (see below for more detailed description of routes of administration).

#### Example 1: The effect of fish oil ( $\omega$ -3 supplement) on fertility of turkeys

##### Materials and Methods

Young tom turkeys were fed a basal diet containing 2830 kcal ME/kg, 9.5% protein, 2.95% fat (mostly from the ingredients and only 0.5% added poultry oil). When they entered maturity, two groups, of 15 birds each, were assigned to the experimental groups, while the rest continued on the basal diet (Control). The feed of group 2 was supplemented with 2.5 - 4.0 % soybean oil, a common fat source in poultry feeds. The feed of group 3 was enriched by ingredients supposed to affect cell membranes' physical

characteristics (2.5 - 4.0 % fish oil. experimental diet). The group on the experimental diet received an addition of 50 per cent more vitamin E. Throughout the three months of the experiment, sperm was collected and assayed, from the initiation of alimentation and twice a week thereafter.

5 Sperm concentration was counted using a Mekler chamber and computerised system. Motility was assayed three hours after collection, diluted 1:1 with a commercial diluter (GOMET, 11) and kept at a temperature between 16 and 22°C. The evaluation was done using a microscope or computerised system. Membrane integrity was determined using fluorescent staining (7.5µg/ml carboxy fluorescent diacetate (CFDA)) and a  
10 fluorescent microscope. The stained fluorescent cells were counted and divided by the non-stained cells. The computerised system for evaluating sperm motility (SMI-sperm motility index) and the results from the fluorescent intensity were expressed as normalised percentage from the control group. Fertility was expressed as the percentage of the fertile eggs obtained under regular agricultural conditions.

## 15 Results

The addition of soybean oil and fish oil increased the concentration of sperm by 25 and 31% respectively compared to the control (FIG 1(1)). The effect of adding soybean oil was moderate compared to adding  $\omega$ -3 fatty acids.

The positive effect of adding soybean oil on the motility was moderate as  
20 compared to that of the experimental diet. The effect of the  $\omega$ -3 supplement (Experimental diet) on motility was more than 3 fold higher than the soy group and approximately 120 times higher than that of the control group (FIG 1(11)).

The viability of the sperm is expressed in two ways, sperm motility index and membrane integrity. The sperm motility index increased relative to the control group with  
25 both soybean oil and the experimental diet. The increase was markedly higher using the experimental diet rather than with the soybean oil. Membrane integrity was only slightly better in the experimental diet group than in the soybean oil one (FIG 1(11)).

It was seen from the fatty acid profile of tom turkeys' sperm that there was a significantly greater amount of 22:6 omega 3 fatty acid in the group fed the commercial  
30 diet with the  $\omega$ -3 supplement, over the group only fed the commercial diet and the group

fed the commercial diet with soybean supplement (FIG 2).

Fertility was calculated as a percentage of fertile eggs from total number of eggs incubated. Between 26.3.98 and 30.4.98 the average fertility was 84.75% in the experimental diet group and 81.55% in the control group. This is an improvement of 3.2%. During this period 57,100 eggs from the control group were incubated. An increase of 3.2% which was obtained by the use of the experimental diet could increase the number of fertile eggs by 1827.

Example 2: Dietary manipulation with fish oil ( $\omega$ -3 supplement) to increase fertility in avian species

Turkey toms: A control group of about 170 toms were fed commercial feed. Fifteen toms were fed for 10 weeks with a diet supplemented by 1.5% fish oil, 7.5% linseeds and 50 mg/kg vitamin E (treatment group). The diet was formulated to give an energy:protein ratio similar to that of the control group. Toms were screened periodically for semen volume, sperm concentration, motility and lipid profile.

Turkey hens: A control group of 7000 hens were fed commercial feed. One hundred female turkey hens were fed for ten weeks (between week 15<sup>th</sup> and 25<sup>th</sup> of lay) with the same supplement as described for toms (treatment group). Laying rate, fertility and embryo mortality (on day 10 of incubation) were recorded. Eggs were stored before incubation for 4, 17 and 28 days at 16°C.

Results

Turkey toms: The lipid profile of the tom's semen showed an increase in the level of DHA from 0.2% in the control group compared to 2.5% in the treated group. The semen volume increased in the treatment group by 55% and the sperm concentration increased by 25% (figure 3). These results show an increase of 60% in the total sperm number in the treatment group as compared to the control one.

Turkey hens: The results in the female hens are shown in table 1. The percentage of fertile eggs was very high in this experiment both in the treatment group and control group (between 94-97%) with no differences between groups. Due to the high fertility in the control group, the results were less pronounced. However, embryo mortality, recorded

on the 10<sup>th</sup> day of incubation, was 4% higher in the control group than in the treatment group after 28 days of storage. Hatchability was 4% higher in the treatment group after 17 days of storage. An increase of 60% in DHA concentration in the egg yolks and also in the embryonic organs (liver, eyes and brain) was found in the treatment group compared to the control group. In addition laying rate in week 24 was higher in the treatment group than in the control group, 55% in the control compared with 60% in the treatment group.

Table 1: Fertile eggs, live embryos and hatchability after storage of 4 to 28 days of eggs from control and treated groups

Treatment group	Storage	number of eggs	% fertility	live embryos	hatchability
control	4 days	11304	94	95	94
$\omega$ -3	4 days	200	96	95	93
control	17 days	188	93	84	84
$\omega$ -3	17 days	188	93	85	88
control	28 days	166	94	35	-
$\omega$ -3	28 days	162	94	39	-

These results show that the  $\omega$ -3 supplement increased fertility in both the toms and hens.

### Example 3: Effect of $\omega$ -3 on the fertility of animals from the ovine species

Rams: Six rams with low fertility and low concentration of  $\omega$ -3 fatty acids were used in this experiment. The rams were used as both the control and treatment groups. For 3 months the rams received commercial feed (control group). Semen was collected every week and evaluated for volume, sperm concentration, motility and lipid profile.

The rams were then fed for three months with a commercial diet, supplemented with 3% fish oil or with 300g of linseeds per day and 50 mg/kg feed of vitamin E (treatment group). Semen was collected every week and evaluated for volume, sperm concentration, motility and lipid profile.

Ewes: Twenty female lambs were fed in addition to the commercial diet, 4% protected fish oil and vitamin E 50 units per kg of feed. The protected fish oil was a Ca salt of the fish oil fatty acids, which allows their by-pass of the rumen. In this way the fish oil is fatty acid protected. An additional eight female lambs were kept as the control.

5 Body weight was recorded weekly, and blood samples were collected four times during the experiment. After four months on the experiment, the animals were slaughtered and the ovaries were evaluated for number of follicles and oocytes per ovary.

### Results

Rams: A small difference was observed in the level of DHA in the sperm, as a result of feeding the supplemented diet. DHA level was increased from 27% before starting the treatment to 29% in the group supplemented with linseeds and from 27.5 to 30.5% respectively in the group supplied with fish oil. Semen characteristics were hardly affected by either of the supplements, except for one ram, which received fish oil as a supplement and its sperm concentration and motility increased by 60%.

15 Ewes: No differences in the body weight was found between the ewes which were fed with protected fish oil or the control group. However, the lipid profile showed differences between the treatment and control groups. The level of EPA and DHA in the blood plasma was 0.83% in the treated group compared with 0.39% in the control group. Moreover, the number of follicles and oocytes increased in the treatment animals from 14.5 to 17.4 per ovary and from 10.8 to 14.7 per ovary, respectively. In addition, the number of high quality oocytes (grade 1-2) was higher (74%) in the treatment group than in the control group ( $P < 0.001$ , 57%).

### Example 4: Effect of $\omega$ -3 on the fertility of animals from the bovine species

25 Four young bulls with low semen quality were fed for four months a commercial diet, supplemented with 4% protected fish oil and vitamin E, 50 units per kg feed. An additional four bulls with high semen quality and one with low semen quality were used as control, being fed a commercial feed. Semen samples were taken weekly and evaluated for sperm concentration and motility using a sperm motility analyser (SMI).

### Results

30 The results are shown in figure 4. After 1.5 months on the treatment diet, the

sperm concentration and motility of these bulls increased from less than 900 million/ml to more than 1.2 billion/ml and from less than 20% to more than 70% respectively.

It was therefore, shown that the  $\omega$ -3 supplement increased fertility in the bulls.

5 Example 5: Effect of  $\omega$ -3 on human fertility - Preliminary human trials

Evidence has been mounting in recent times of declining male fertility among humans, as well as among domesticated animals. 27 male human subjects with low fertility were used in the trial. For one month, the subjects received the following daily regimen. The subjects received capsules of  $\omega$ -3 (Omagene forte, 2 x 500mg). These  
10 capsules contained EPA and DHA. In addition the subjects were fed a diet with a high concentration of oily fish such as mackerel, sardines and herring (100g), eggs containing  $\omega$ -3, and canola oil (20g).

Results

Two of the subjects, who initially had low levels of  $\omega$ -3 in their sperm and whose  
15 sperm quality was inferior showed a marked improvement after one month on the aforementioned treatment (results not shown). The increased fertility of the sperm of one subject has been confirmed by a pregnancy.

The present invention using dietary manipulation with  $\omega$ -3 has therefore been shown to increase fertility in human males.

20

Example 6: Possible methods of use

Examples 1-6 illustrates specific methods of use. A general method of use is now described. 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA) fatty acids can be administered to a subject in a number of ways, which  
25 are well known in the art. Hereinafter, the term 'subject' refers to the turkey or other poultry, lower mammal or human to whom 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA) fatty acids were administered. For example administration may be done orally, topically or parentally.

Compositions for oral administration, which is the preferred route of  
30 administration, is a form that can be added to feed during feeding or before and include

powders or granules, suspensions or solutions, in non-aqueous media, sachets, capsules or tablets.  $\omega$ -3 fatty acids can be used in a protected form to prevent degradation, such as in a protective fat. Thickeners, diluents, flavourings, vitamins dispersing aids, emulsifiers or binders may be desirable.

5        Dosing is dependent on the responsiveness of the subject to 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA) fatty acids. The amount received by the subject is controlled. For example as part of a diet it would be administered at the time the subject ate, or alternatively as a pill, the dose and frequency of dosing would be dependent on the responsiveness of the subject. Persons of ordinary  
10    skill in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

Example 7: General method of treatment of sub-fertility

The following example illustrates a method of treating sub-fertility, characterised  
15    by the effect of 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA) fatty acids on the fatty acid composition of sperm membranes and is not intended to be limiting.

The method includes the step of administering 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA) fatty acids in an acceptable form  
20    e.g., by adding to the feed of the subject. 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA) fatty acids are administered according to an effective dosing methodology, preferably until a predefined endpoint is reached, such as an optimum increase in fertility.

While the invention has been described with respect to a limited number of  
25    embodiments, it will be appreciated that many variations and other applications of the invention may be made.



## APPENDIX

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What is claimed is:

1. A dietary supplement to increase fertility in an animal, the supplement comprising a  $\omega$ -3 fatty acid-containing component.

2. The dietary supplement of claim 1, wherein said animal includes an agriculturally useful animal.

3. The dietary supplement of claim 1, wherein said animal includes poultry.

4. The dietary supplement of claim 1, wherein said animal includes a turkey.

5. The dietary supplement of claim 1, wherein said animal includes a lower mammal.

6. The dietary supplement of claim 1, wherein said animal includes an animal from the ovine species.

7. The dietary supplement of claim 1, wherein said animal includes an animal from the bovine species.

8. The dietary supplement of claim 1, wherein said animal includes a non-mammalian aquatic animal.

9. The dietary supplement of claim 1, wherein said animal includes a human.

10. The dietary supplement of claim 1, wherein said  $\omega$ -3 fatty acid containing component includes 18:3 omega 3 (LNA).

11. The dietary supplement of claim 1, wherein said  $\omega$ -3 fatty acid containing

component includes 20:5 omega 3 (EPA).

12. The dietary supplement of claim 1, wherein said  $\omega$ -3 fatty acid containing component includes 22:6 omega 3 (DHA).

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13. The dietary supplement of claim 1, wherein said  $\omega$ -3 fatty acid containing component includes 22:5 omega 3 (DPA).

14. The dietary supplement of claim 1, wherein said  $\omega$ -3 fatty acid containing component includes a mixture of at least two of the fatty acids, 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA).

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15. The dietary supplement of claim 1, wherein said  $\omega$ -3 fatty acid containing component includes fish oil.

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16. The dietary supplement of claim 1, wherein said  $\omega$ -3 fatty acid containing component is selected from the group consisting of fish meal, linseeds and purslane.

17. The dietary supplement of claim 1, wherein said  $\omega$ -3 fatty acid containing component is a single cell oil source.

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18. The dietary supplement of claim 1, wherein said fertility is decreased embryo mortality.

19. The dietary supplement of claim 1, wherein said fertility is oocyte fertility.

25

20. The dietary supplement of claim 1, wherein said fertility is number of eggs.

21. The dietary supplement of claim 1, wherein said fertility is quality of eggs.

30

22. The dietary supplement of claim 1, wherein said fertility is hatchability.
23. The dietary supplement of claim 1, wherein said fertility is number of follicles.
- 5 24. The dietary supplement of claim 1, wherein said fertility is determined according to sperm concentration.
25. The dietary supplement of claim 1, wherein said fertility is determined  
10 according to sperm viability.
26. The dietary supplement of claim 1, wherein said fertility is determined according to sperm motility.
- 15 27. A method for increasing fertility in an animal, comprising feeding said animal a  $\omega$ -3 fatty acid containing component, in an amount effective to increase fertility.
28. The method of claim 27, wherein said fertility is determined according to sperm concentration.
- 20 29. The method of claim 27, wherein said fertility is determined according to sperm viability.
30. The method of claim 27, wherein said fertility is determined according to  
25 sperm motility.
31. The method of claim 27, wherein said fertility is selected from the group consisting of laying rate, embryo mortality, number and quality of eggs, number of follicles or oocyte fertility.
- 30 32. The method of claim 27, wherein said animal includes an agriculturally

useful animal.

33. The method of claim 27, wherein said animal includes poultry.

5 34. The method of claim 27, wherein said animal includes a turkey.

35. The method of claim 27, wherein said animal includes a lower mammal.

36. The method of claim 27, wherein said animal includes a non-mammalian  
10 aquatic animal.

37. The method of claim 27, wherein said animal includes a human.

38. The method of claim 27, wherein said  $\omega$ -3 fatty acid containing component  
15 is selected from the group consisting of whole fish oil, 18:3 omega 3 (LNA), 20:5 omega  
3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA) fatty acids and mixtures  
thereof.

39. The method of claim 27, wherein said  $\omega$ -3 fatty acid containing component  
20 is of animal origin.

40. The method of claim 27, wherein said  $\omega$ -3 fatty acid containing component  
is of plant origin.

25 41. The method of claim 27, wherein said  $\omega$ -3 fatty acid containing component  
is of single cell oil origin.

42. The method of claim 27, wherein said  $\omega$ -3 fatty acid containing component  
is selected from the group consisting of fish meal, linseeds and purslane.

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43. A method for increasing fertility in an animal, by introducing a  $\omega$ -3 fatty acid containing component into animal gametes, comprising feeding said animal a  $\omega$ -3 fatty acid containing component, in an amount effective to increase fertility.

5 44. The method of claim 43, wherein said animal includes an agriculturally useful animal.

45. The method of claim 43, wherein said animal includes poultry.

10 46. The method of claim 43, wherein said animal includes a turkey.

47. The method of claim 43, wherein said animal includes a lower mammal.

15 48. The method of claim 43, wherein said animal includes a non-mammalian aquatic animal.

49. The method of claim 43, wherein said animal includes a human.

20 50. The method of claim 43, wherein said animal gamete is a sperm.

51. The method of claim 43, wherein said animal gamete is an oocyte.

25 52. The method of claim 43, wherein said  $\omega$ -3 fatty acid containing component is selected from the group consisting of whole fish oil, 18:3 omega 3 (LNA), 20:5 omega 3 (EPA), 22: 5 omega 3 (DPA) and 22: 6 omega 3 (DHA) fatty acids and mixtures thereof.

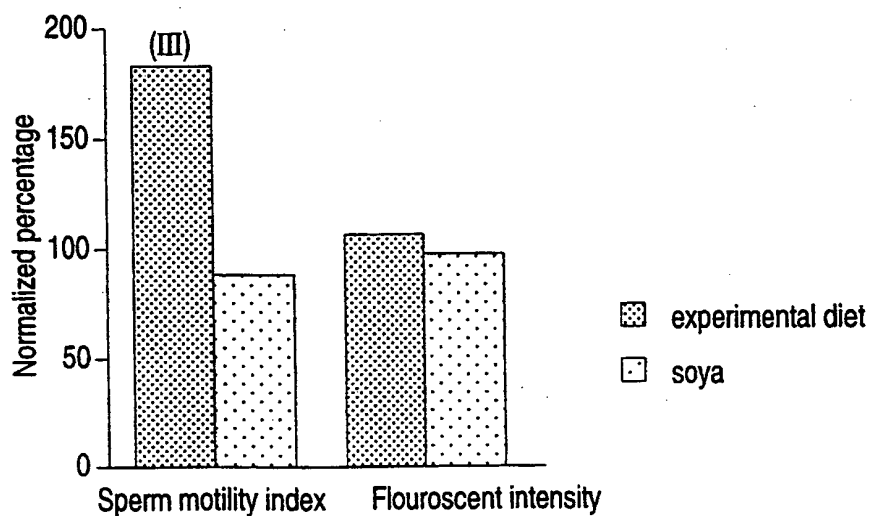
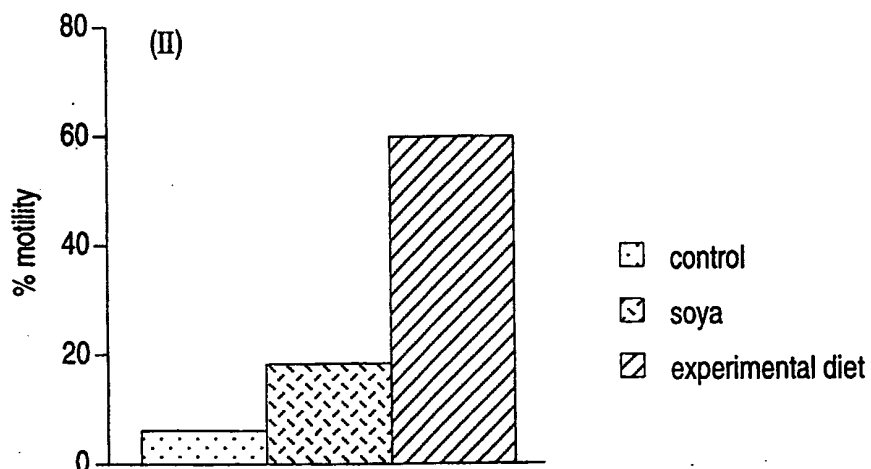
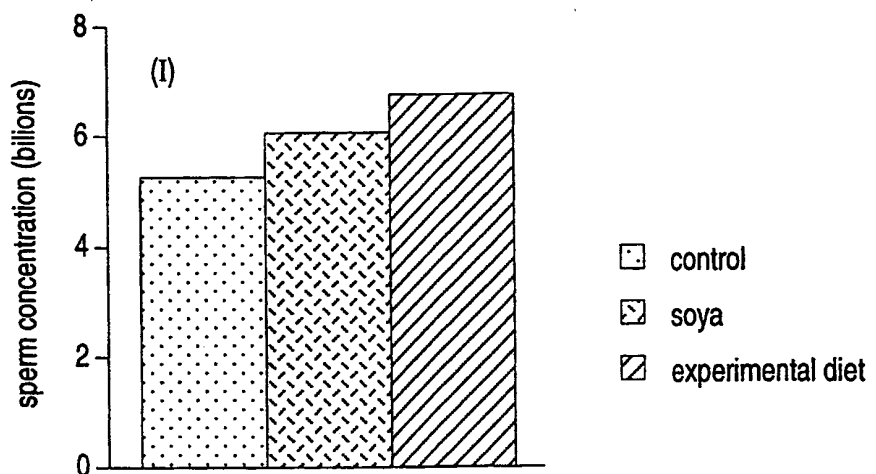
30 53. The method of claim 43, wherein said  $\omega$ -3 fatty acid containing component is of animal origin.

54. The method of claim 43, wherein said  $\omega$ -3 fatty acid containing component is of plant origin.

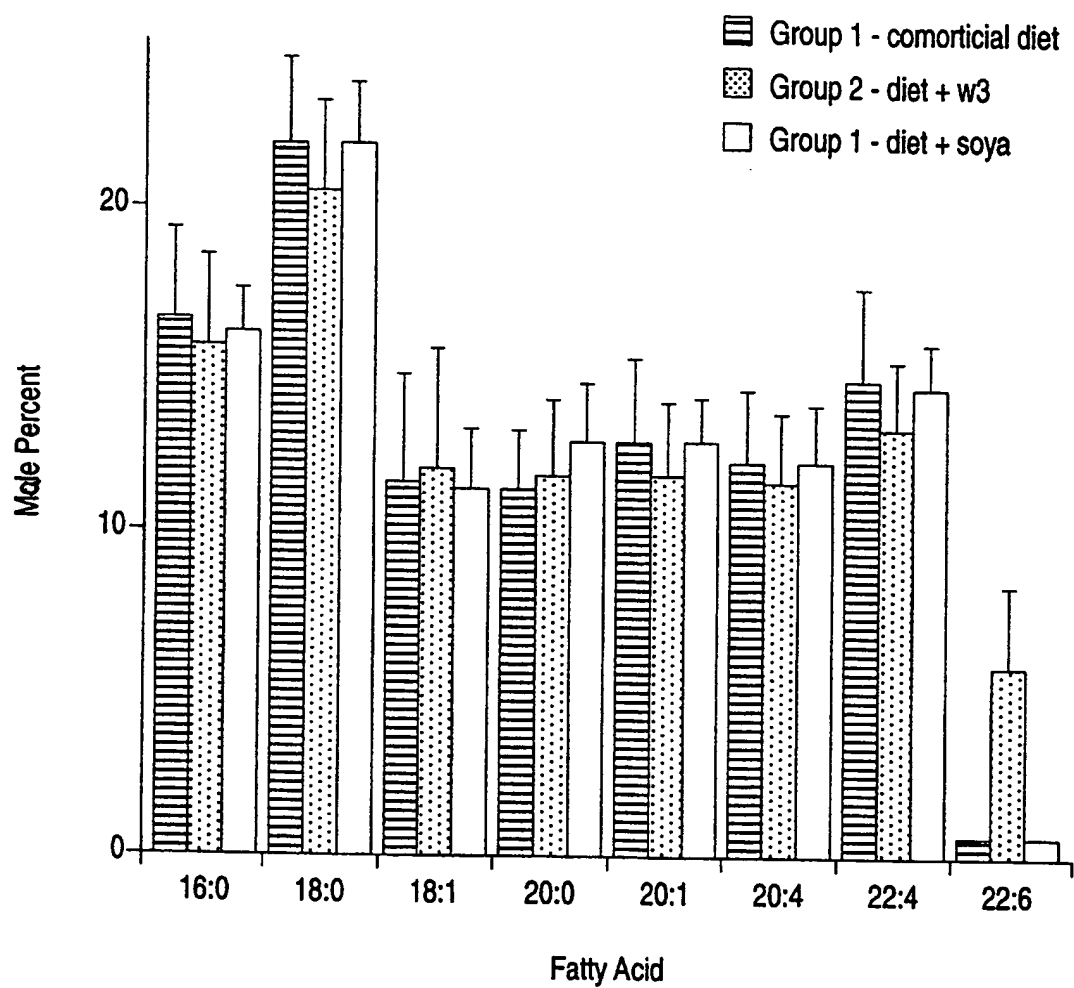
55. The method of claim 43, wherein said  $\omega$ -3 fatty acid containing component  
5 is of single cell oil origin.

56. The method of claim 43, wherein said  $\omega$ -3 fatty acid containing component is selected from the group consisting of fish meal, linseeds and purslane.

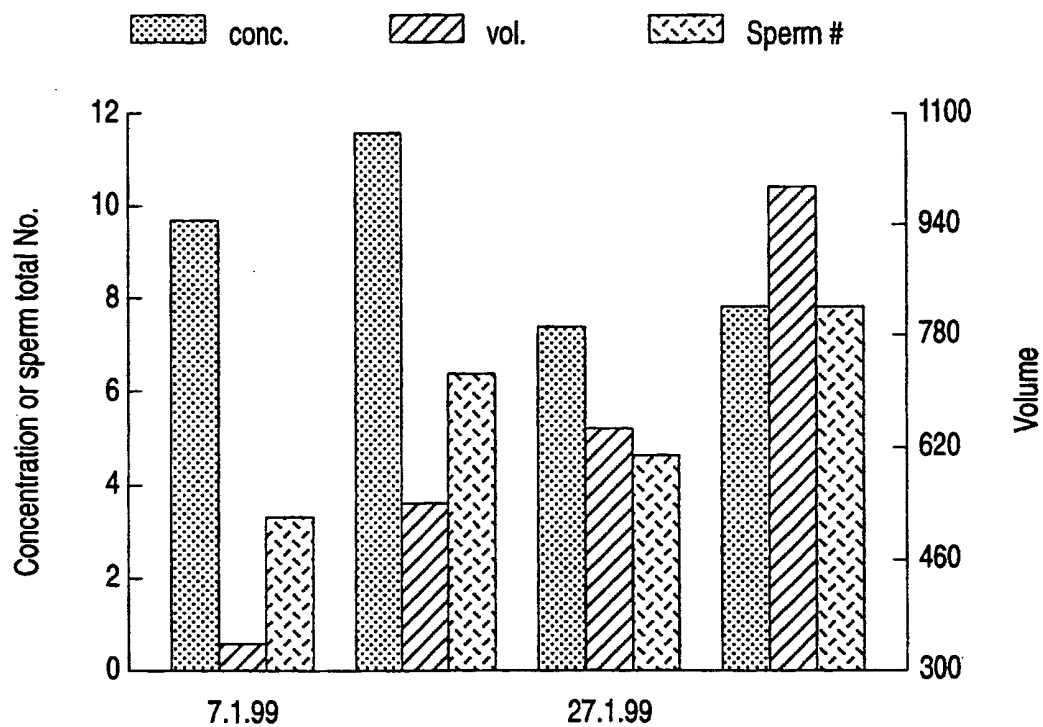
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**FIGURE 1.** The Effect of dietary lipids on toms sperm concentration (I), motility (II), and viability (III)



**FIGURE 2.** Fatty acid profile of tom's sperm

**FIGURE 3.** Effect of dietary w-3 fatty acids on volume, concentration and number of sperm



**FIGURE 4.** The Effect of w-3 fatty acids on sperm motility and concentration in bulls

